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Award Number: DAMD17-03-1-0537

TITLE: The Effects of Diesel Exhaust and Stress on the Acute Phase Response and Symptoms in the Chemically Intolerant

PRINCIPAL INVESTIGATOR: Nancy Fiedler, Ph.D.

CONTRACTING ORGANIZATION: University of Medicine and Dentistry of New Jersey
Newark, NJ 07107

REPORT DATE: August 2007

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
01-08-2007	Annual	1 Aug 2006-31 Jul 2007

4. TITLE AND SUBTITLE
The Effects of Diesel Exhaust and Stress on the Acute Phase Response and in the Chemically Intolerant

5a. CONTRACT NUMBER

5b. GRANT NUMBER
DAMD17-03-1-0537

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Nancy Fiedler, Ph.D.

E-Mail: nfiedler@eohsi.rutgers.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Medicine and Dentistry of New Jersey
Newark, NJ 07107

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT Exposures to diesel exhaust (DE) and other petrochemical combustion products were the exposures reported by the greatest percentage of all Gulf War veterans (GWV). Along with diesel exhaust and other chemical exposures, psychological stress has been implicated in the onset of unexplained symptoms such as chemical sensitivity among GWV. The purpose of the proposed study is to test a model for chemical sensitivity in GWV, in which simultaneous acute exposures to DE and psychological stress cause increased symptoms via the acute phase response (APR), in susceptible individuals. Individuals who are low or high in the susceptibility factor of chemical intolerance (CI) will be exposed to DE either with or without a public speaking task, an acute psychological stressor. 100 subjects have completed the protocol. Preliminary data indicates that relative to clean air, subjects report a small increase in symptoms following the onset of diesel exposure. Analysis of blood cell counts and differentials and soluble markers reveal the reliability of the analytic techniques and compare favorably to normative reference ranges. Analysis of induced sputum cell differential counts showed that a minority of subjects (N=19) were able to produce two adequate sputum samples. Tests of study hypotheses are pending completion of assays for soluble markers in collected blood, sputum and nasal lavage samples, and ongoing statistical analysis and interpretation of results.

15. SUBJECT TERMS
Diesel exhaust, chemical sensitivity, stress, acute phase response, symptoms, inflammation, chemical intolerance

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			**19b. TELEPHONE NUMBER** *(include area code)*
U	U	U	UU	11	USAMRMC

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INTRODUCTION

Exposure to DE, kerosene, and/or other petrochemical vapors and incomplete combustion products was the exposure reported by the greatest percentage of all GWV (Kang et al., 2000) and was, along with numerous other self-reported exposures, associated with increased risk of various medically unexplained symptoms, including chemical sensitivity symptoms (Spencer, 2001; Fiedler et al. , Kipen et al., Wolfe et al., 2002). Many airborne chemical exposures may cause irritation of the eyes and respiratory tract, and recent controlled exposure studies have shown that DE (DE) can cause an acute inflammatory response in the respiratory tract. Such chemical exposure alone, however does not satisfactorily account for the multiple unexplained symptoms of veterans, and the stress of deployment and war has been cited as a contributory factor (Presidential Advisory Committee on GWV' Illnesses, 1996; Institute of Medicine). We are proposing to test a model for low-level chemical sensitivity in GWV (GWV's), in which simultaneous, acute exposure to DE and psychological stress cause symptoms through a common pathway, the acute phase response (APR). As demonstrated in experimental studies, the APR is activated independently by both psychological stress and local airway inflammation caused by acute inhalation of DE and other air contaminants. A main objective of the proposed study is to test the effects of an interaction between acute psychological stress and airway inflammation due to DE, an interaction that has not been studied previously. We will test several hypotheses suggested by this model, namely that: 1) Acute inhalation exposure to DE will cause a measurable local inflammatory response in the upper and lower respiratory tract, 2) Acute DE exposure alone will cause an APR, 3) An acute psychological stressor alone will cause an APR , 4) Simultaneous exposure to DE and an acute psychological stressor will interact additively or synergistically to enhance the APR, and 5) An enhanced APR will be associated with increased symptoms.

While exogenous exposures, such as DE and acute psychological stress, may well contribute to the symptoms of Gulf war illness, many veterans had no apparent health effects, suggesting that some individual psychological or physiological differences may have contributed to the response, and implicating some form of increased susceptibility. Studies of symptomatic GWV, however, cannot adequately test the interaction of susceptibility and exposure due to the potentially confounding effects of illness. Therefore, we also propose to test the effects of exposure to DE and stress among healthy subjects who are low or high in the susceptibility factor of self-reported chemical intolerance, a phenomenon of undefined mechanism.

BODY

Goals and Objectives for Year 4:

August, 2006– July, 2007

A. Complete exposure sessions, data coding, and entry for remaining subjects (total 100)

Subject participation was completed in April of 2007. No major adverse effects were reported. Data has been coded and entered for results of various assays, as specified in original proposal, and are summarized as follows.

Exposure analysis

Exposure analyses were performed as proposed. Detailed analysis of the diesel exhaust components (from one exposure condition) and a summary of analysis of as sample of the diesel exhaust (n=20) and clean air control (n=22) exposure conditions is provided below.

Characterization of diluted exhaust (DE 300 µg/m3) from the diesel generator operated at 100% load (01/26/2007)

Analyte	Units	Concentrations (Mean ± SD)
$PM_{2.5}$ Mass	$\mu g/m^3$	314 ± 27.9
PM number	$\#/cm^3$	97715 ± 11665
CO	ppm	4.93 ± 0.86
NO	ppm	6.01 ± 1.13
NO_X	ppm	6.18 ± 1.13
Elemental Carbon (EC)	$\mu g/m^3$	144
Organic Carbon (OC)	$\mu g/m^3$	94.6
Total Carbon (TC)	$\mu g/m^3$	238
PAH:		
Naphthalene	ng/m^3	2.48
Acenaphthene	ng/m^3	3.99
Fluorene	ng/m^3	11.3
Phenanthrene	ng/m^3	29.5
Anthracene	ng/m^3	2.05
Fluoranthene	ng/m^3	5.05
Pyrene	ng/m^3	87.2
Benzo(a)anthracene	ng/m^3	50.7
Chrysene	ng/m^3	12.2

Benzo(b)fluoranthene	ng/m^3	1.42
Benzo(k)fluoranthene	ng/m^3	0.47
Benzo(a)pyrene	ng/m^3	0.12
Dibenzo(a,h)anthracene	ng/m^3	0.24
Benzo(ghi)perylene	ng/m^3	0.73
Indeno(1,2,3-cd)pyrene	ng/m^3	ND
Aldehydes:		
Formaldehyde	**µ**g/m^3	347
Acetaldehyde	**µ**g/m^3	153
Acetone	**µ**g/m^3	58.1
Acrolein	**µ**g/m^3	ND
Propionaldehyde	**µ**g/m^3	10.6
Methyl-Ethyl Ketone	**µ**g/m^3	0.11
Crotonaldehyde	**µ**g/m^3	ND
Butyraldehyde	**µ**g/m^3	3.85
Benzaldehyde	**µ**g/m^3	6.24
Isovaleraldehyde	**µ**g/m^3	ND
Valeraldehyde	**µ**g/m^3	8.72
o-Tolualdehyde	**µ**g/m^3	ND
m-/p-Tolualdehyde	**µ**g/m^3	3.83
Hexaldehyde	**µ**g/m^3	ND
2,5-Dimethylbenzaldehyde	**µ**g/m^3	ND

Note: ND means Not Detected.

Summary of CEF exposure conditions for the sessions performed from August 15, 2006 to March 20, 2007

		$PM_{2.5}$ Mass (µg/m3)	PM number (#/cm^3)	CO (ppm)	NO (ppm)	NO_2 (ppm)
Diesel	Mean	277	69017	4.64	4.30	0.13
Exposure	SD	18.7	17319	0.62	0.76	0.034
(N=20)	Range	239-306	47930-104988	2.89-5.70	3.40-6.50	0.069-0.20
Clean Air	Mean	6.15	2546	0.99	0.010	0.008
(N=22)	SD	5.09	1380	0.21	0.018	0.007
	Range	1.10-23.0	745-5898	0.72-1.43	0.001-0.085	0-0.027

Blood cell counts

Flow cytometry analyses were completed on blood samples from the various time points (pre-exposure, pre-stress, post-stress, 6 hr and 24 hr post exposure) on the 100 subjects,

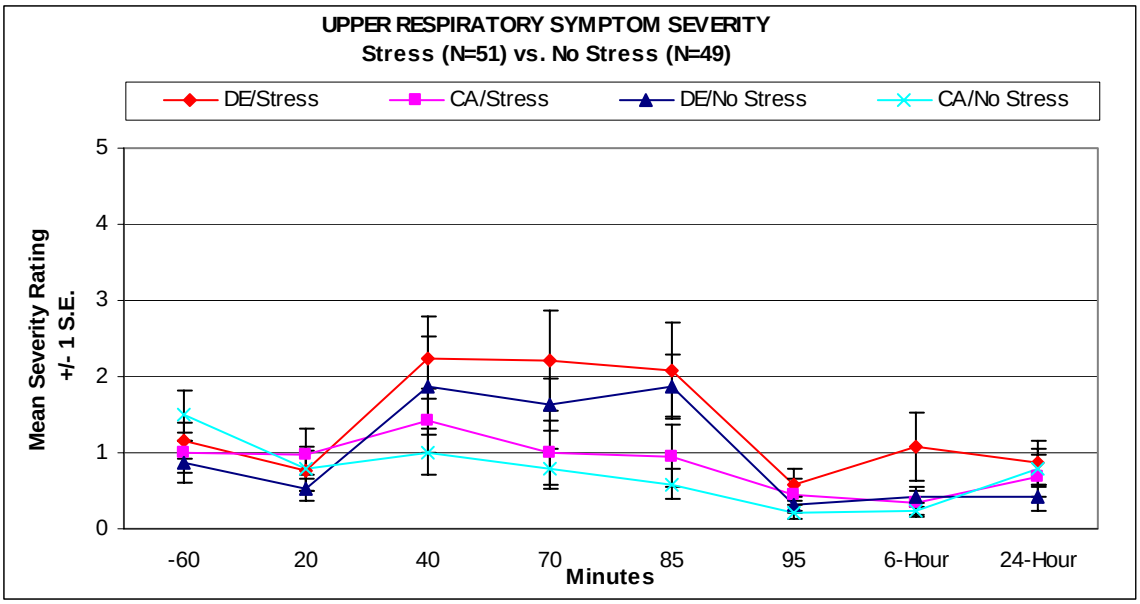
including total leukocytes, neutrophils, lymphocytes, CD4 cells, CD8 cells, and natural killer cells. Additional blood count parameters include complete blood count (CBC) data (red blood cell count, mean corpuscular volume, hematocrit, hemoglobin, platelet count) and white blood cell differential (neutrophils, lymphocytes, eosinophils, basophils, monocytes) from blood samples at pre-exposure, 6hr, and 24 hr.

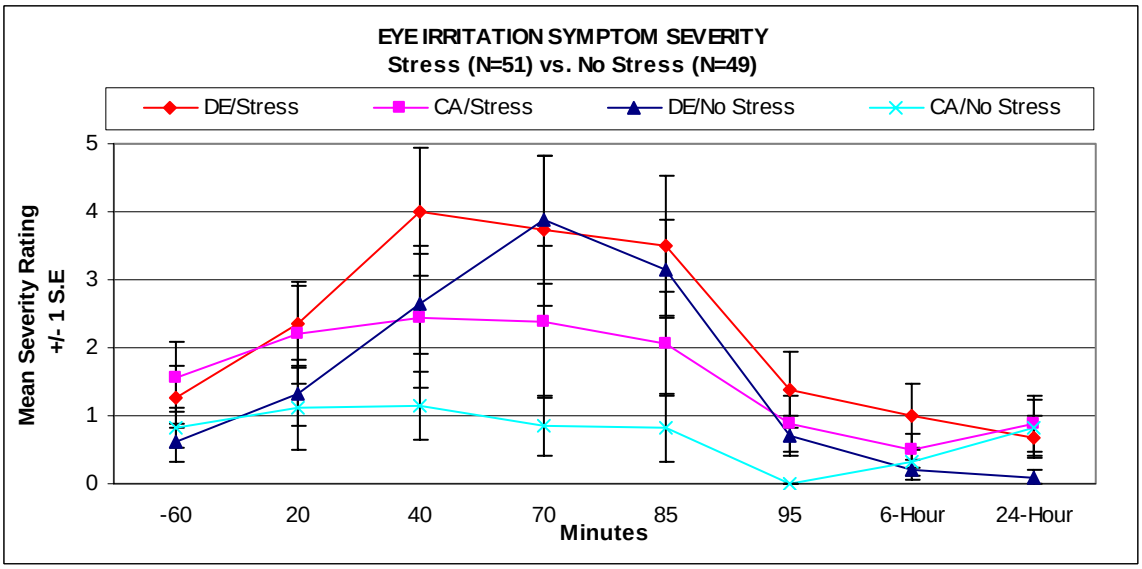
Soluble blood parameters
Plasma from blood samples at the various proposed time points have been assayed for interleukin 6 (IL-6), c-reactive protein (CRP), fibrinogen, and cortisol.

Sputum cell counts
Based on hemocytometer counts of cells in sputum samples, 42 subjects met criteria for having two adequate sputum samples (having adequate visible mucus "plugs" that were selected from the sample, minimal oral contamination(<25% squamous cells) and sufficient sample size (>100,000 total cells). However due to apparent undercounting of squamous cells on the hemocytometer, differential counts on cell cytospins resulted in only 19 subjects (38 samples) having adequate samples. We are focusing our initial analysis and interpretation on these 38 samples, due to the strength of within-subject comparisons, but may expand the assays to include subjects with single adequate samples. Also, since the criteria for an adequate sputum sample are somewhat subjective, we may consider using less strict criteria.

Soluble makers in sputum
The 19 pairs of adequate samples (6 h post control and diesel exhaust exposures), are being assayed for IL-8, IL-6 and IL-1 cytokines. These assays are in process.

Symptoms
Data on 31 symptoms has been coded and entered for all subjects. Symptoms have been categorized in subsets (eg upper respiratory, lower respiratory, eye irritation, acute phase response, central nervous system, etc) for subsequent analysis. As expected, preliminary analysis has shown modest increase in upper respiratory symptom severity and eye irritation symptom severity at various time points during exposure, comparing diesel exhaust (DE) sessions to clean air (CA) control. These differences resolved at later time points (See preliminary graphs below, in which time 0 minutes is entering exposure facility and exposure commenced at 30 minutes).





Stress reactivity

5-minute segments of the continuous intra-session ECG data have been cleaned and analyzed with heart-rate variability software to yield 5-minute mean heart rate, standard deviation of normal-normal intervals (SDNN), RMSSD (root mean squatter of normal-normal intervals), pN50 (difference between normal-normal >50 ms), and high and low frequency heart rate variability. The analyzed segments were identified as pre-exposure, pre-stress, and post-stress (end of exposure), time points. These data will be combined with cortisol, natural killer cell counts, and symptom reporting as indices of cardiovascular, immune, and psychological stress reactivity that will be used to test for interactions between individual reactivity and the main effects of exposure and experimental stress on symptoms and physiological outcomes.

Statistical analysis
Statistical summary and analysis for main effects of diesel exhaust exposure and stress on the symptom and physiological outcomes is ongoing. Full analysis and interpretation of results will be completed for final report scheduled to be completed by April 1, 2008.

KEY RESEARCH ACCOMPLISHMENTS

"Non-invasive measures of oxidative stress and inflammatory responses to diesel exhaust particles in human respiratory epithelium. Abstract presentation at the American Thoracic Society, 2005.

"The effects of diesel exhaust and stress on systemic inflammation and the acute phase response." Abstract presentation at the "Mechanisms of Action of Inhaled Fibers, Particles, and Nanoparticles in Lung and Cardiovascular Disease" conference, sponsored by NIEHS and NIOSH, on October 25-28, 2005.

"The effects of diesel exhaust and stress on the acute phase response and symptoms in the chemically intolerant" Symposium: environmental modulation of neurotoxicants in military-relevant environments". International Neurotoxicology Conference, September, 2006.

"Effects of diesel exhaust and stress on systemic inflammation and the acute phase response in humans: Acute peripheral leukocyte counts." Presented at EOHSI Research Day, May, 2006.

"Diesel exhaust and psychological stress: Redistribution of human peripheral blood leukocytes." Presented at the American Thoracic Society (ATS) 2006 International Conference, San Diego, CA, May 22, 2006.

"Combined effects of diesel exhaust and psychological stress on inflammation and the acute phase response in normal humans."The Role of Air Pollutants in Cardiovascular Disease, EPA and NIEHS-sponsored meeting at USEPA, RTP, October 12-13, 2006.

"Effects of diesel exhaust and stress on systemic inflammation and the acute phase response in humans." Presented at the American Thoracic Society (ATS) 2006 International Conference, San Francisco, CA, May 19-24, 2007

REPORTABLE OUTCOMES

Tests of study hypotheses are pending completion of remaining assays and statistical analysis of results, which are in process. However, based on the development of our diesel exposure capabilities, the following projects have been initiated to augment outcome measures in the current study as well as additional studies using similar exposure paradigms and outcome measures.

Kipen, H (principal investigator). Responses to Fresh Aerosol in Susceptible Subjects, funded by EPA, R832144, $1,521,398. The purpose of this study is to evaluate cardiovascular effects of diesel exhaust. Subjects will be exposed to 200 $\mu g/m^3$ $PM_{2.5}$ for 2 hours and platelet activation, endothelial dysfunction, and pulmonary inflammation through induced sputum will be measured.

"Noninvasive Measures of Oxidative Stress and Inflammatory Responses to Diesel Exhaust in Human Respiratory Epithelium." Laumbach RJ (PI). 12/2004-6/2005 Funded by NIOSH ERC Pilot project. $10,000. This study examines molecular markers of oxidative stress and inflammation in nasal respiratory epithelium samples from human subjects after controlled exposure to diesel exhaust. The goal is to develop and validate a new noninvasive techniques for studying responses to diesel exhaust in controlled exposure and epidemiology studies.

"Mechanisms of Responses to Diesel Exhaust and Stress." Laumbach RJ (PI). K08 Career Development, NIEHS. $125,000 x 5 years, This career award will provide support for development of the PI's capability to perform complementary studies of responses to diesel exhaust and stress in relevant animal models and human subjects.

CONCLUSIONS

After significant delays in subject recruitment deferral of review by the DOD IRB, we have completed the subject recruitment and controlled exposure phase of the study in April of 2007. Exposure characterization has been completed. Blood and sputum cell counts have been completed, and most of the assays for soluble markers in blood and sputum have been completed. Tests of study hypotheses are pending completion of assays for soluble markers in collected blood, sputum and nasal lavage samples, and ongoing statistical analysis and interpretation of results.

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